

SARS-CoV-2 virologic rebound with nirmatrelvir-ritonavir therapy

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Abstract

Objective: To compare the frequency of replication-competent virologic rebound with and without nirmatrelvir-ritonavir treatment for acute COVID-19. Secondary aims were to estimate the validity of symptoms to detect rebound and the incidence of emergent nirmatrelvir-resistance mutations after rebound.

Design: Observational cohort study.

Setting: Multicenter healthcare system in Boston, Massachusetts.

Participants: We enrolled ambulatory adults with a positive COVID-19 test and/or a prescription for nirmatrelvir-ritonavir.

Exposures: Receipt of 5 days of nirmatrelvir-ritonavir treatment versus no COVID-19 therapy.

Main Outcome and Measures: The primary outcome was COVID-19 virologic rebound, defined as either (1) a positive SARS-CoV-2 viral culture following a prior negative culture or (2) two consecutive viral loads ≥ 4.0 log₁₀ copies/milliliter after a prior reduction in viral load to < 4.0 log₁₀ copies/milliliter.

Results: Compared with untreated individuals (n=55), those taking nirmatrelvir-ritonavir (n=72) were older, received more COVID-19 vaccinations, and were more commonly immunosuppressed. Fifteen individuals (20.8%) taking nirmatrelvir-ritonavir experienced virologic rebound versus one (1.8%) of the untreated (absolute difference 19.0% [95%CI 9.0-29.0%], P=0.001). In multivariable models, only N-R was associated with VR (AOR 10.02, 95%CI 1.13-88.74). VR occurred more commonly among those with earlier nirmatrelvir-ritonavir initiation (29.0%, 16.7% and 0% when initiated days 0, 1, and ≥ 2 after diagnosis, respectively, P=0.089). Among participants on N-R, those experiencing rebound had prolonged shedding of replication-competent virus compared to those that did not rebound (median: 14 vs 3 days). Only 8/16 with virologic rebound reported worsening symptoms (50%, 95%CI 25%-75%); 2 were completely asymptomatic. We detected no post-rebound nirmatrelvir-resistance mutations in the NSP5 protease gene.

Conclusions and Relevance: Virologic rebound occurred in approximately one in five people taking nirmatrelvir-ritonavir and often occurred without worsening symptoms. Because it is associated with replication-competent viral shedding, close monitoring and potential isolation of those who rebound should be considered.

Introduction

Data are conflicting about whether nirmatrelvir-ritonavir (N-R) is associated with virologic rebound (VR).¹⁻⁷ However, precise estimation of VR incidence with and without N-R use has been limited by infrequent and short-term sampling, symptomatic reporting, and absence of culture data.

Methods

The Post-vaccination Viral Characteristics Study (POSITIVES) is a prospective, observational cohort of individuals with acute COVID-19 with longitudinal sampling for viral load, viral culture, and symptom reporting (**supplementary appendix**).^{8,9} Participants are sampled from automated medical record reports in the Mass General Brigham healthcare system on individuals with positive testing or a prescription for COVID-19 therapeutics.

Participants self-collect anterior nasal swabs three times a week for two weeks and weekly thereafter until SARS-CoV-2 viral load testing is persistently undetectable. Specimens are analyzed for SARS-CoV-2 viral load, viral culture, and whole genome sequencing. Participants complete 10-item COVID-19 symptom surveys, graded as absent (0), mild (1), moderate (2), or severe (3), for a maximum total symptom score (TSS) of 30-points. Study physicians complete chart reviews to determine COVID-19 vaccination and treatment history, and immunosuppression status (**STable1**).

We sought to estimate the incidence of virologic rebound, which we defined in individuals with either 1) positive SARS-CoV-2 viral culture following a negative culture or 2) a viral load $\geq 1.0 \log_{10}$ from a prior viral load and $\geq 4.0 \log_{10}$ copies/mL for two consecutive timepoints after a

prior reduction in viral load to $<4.0 \log_{10}$ copies/mL. We selected this outcome as a surrogate for putative transmission risk, based on data relating transmission to replication-competent virus with viral loads $>4.0 \log_{10}$ copies/mL.^{10,11} For a secondary outcome, we redefined VR as a viral load at days 10 and 14 $\geq 2.7 \log_{10}$ and at least $0.5 \log_{10}$ greater than the result at day 5, in order to compare our estimates to the EPIC-HR study, which considered fewer time points and did not incorporate culture methods.¹

Our primary exposure of interest was exposure to N-R therapy. Therefore, we limited analysis to ambulatory participants enrolled after March 2022, when we began recruiting individuals initiating N-R. We also excluded participants without a nasal swab collected >11 days from their first positive COVID-19 test, because approximately 90% of rebound phenomena occur by this time,⁸ and individuals who received N-R for more or less than 5 days. We compared the frequency of VR by N-R use overall and stratified by potential confounders (i.e., immunosuppression, age, sex, and prior COVID-19 vaccinations) using two-sided Fisher's exact tests, and after adjustment for confounders, in logistic regression models. We compared the frequency of VR by timing of N-R initiation, using a non-parametric test of trend. We compared our estimate of VR with the definition used in the EPIC-HR study.¹ We used the Kaplan-Meier survival estimator to depict and compare days to initial and final viral culture negativity, stratified by N-R use and VR, using log-rank testing. We assessed the validity of symptom rebound, as defined by an increase in TSS by 3 or more points from a prior date, and the presence of any symptoms during the rebound period, to detect VR.⁶ Finally, we report the proportion of sequenced viruses before and after VR with mutations in the NSP5 gene encoding

the main protease (M^{pro}) of SARS-CoV-2. Statistical analyses and figure production were conducted with Stata version 16.1 and GraphPad Prism version 9.5.

Ethical Considerations

All study participants provided verbal informed consent. Written consent was waived by the ethics committee, based on the involvement of participants with acute COVID-19 in a minimal risk study. The study procedures were approved by Institutional Review Board and the Institutional Biosafety Committee at Mass General Brigham.

Results

Compared with untreated individuals (n=55), those taking N-R (n=72) were older (57 vs 39 years, P<0.001), received more COVID-19 vaccinations (median 4 vs 3, P<0.001) and were more commonly immunosuppressed (32% vs 9%, P<0.001, **SFig1/STable2**). Fifteen individuals (20.8%) taking N-R experienced VR versus one (1.8%) untreated individual (**Figures 1&2**, absolute difference 19.0% [95%CI 9.0-29.0%], P=0.001). In sub-group analyses, VR was numerically more frequent in all demographic and clinical sub-groups (**Figure 2**). In multivariable logistic regression models including demographic and clinical characteristics, only N-R use remained associated with VR (**STable 3**). There was a trend towards higher rates of VR with earlier N-R initiation (29%, 16.7% and 0% when initiated days 0, 1, and ≥2 after diagnosis, P=0.089, **Figure 2**). When we restricted analyses to three timepoints, as done in the EPIC-HR study, only 3/124 (2.4%) had rebound detected, and 13/16 (81.2%) rebound events were not captured (**Figure 1E-F**). We detected no post-N-R drug resistance mutations in the NSP5 protease gene (**SFig2**).

N-R recipients achieved initial culture conversion sooner than those not treated (**Figure 3/STable4**, $P < 0.001$). However, days to final culture conversion was similar (**Figure 3**, $P = 0.29$) because those experiencing VR had significantly prolonged shedding (median 14 [IQR 13-20] vs 3 days [IQR 2-4], **Figure 3/STable4/STable5**). Only 8/16 with VR reported symptom rebound (50%, 95% CI 25-75%); 2 were totally asymptomatic. Only 8/27 with symptom rebound had VR (30%, 95% CI 14-50%, **SFig3/STable6**).

Discussion

VR with replication-competent viral shedding occurred in approximately 20% of those taking N-R and 2% of those not on therapy. N-R use remained associated with VR after adjustment for demographic and clinical characteristics, such as vaccination and immunosuppression status. Although N-R treated individuals took fewer days to achieve initial culture negativity, time to final culture negativity was similar, due to prolonged shedding of replication-competent virus among those experiencing VR (median 14 vs 3 days). These data support the presence of an N-R-associated virologic rebound phenomenon, which substantially increases the duration of shedding of replication-competent virus and has implications for post-N-R monitoring and isolation recommendations.

We found a higher incidence of VR with N-R use than prior studies. We believe this is due to use of frequent sampling and culture methods to detect VR. When we restricted our analysis to three PCR-based timepoints, as done in prior trials,¹ we detected a 2.4% rate of VR, which approximates prior studies, but notably missed 80% of VR events.

178
179 VR appeared to be less common among those who delayed therapy by 1 or 2 days after their first
180 positive test. This finding, in conjunction with the lack of drug resistance-associated mutations
181 after VR events, promotes hypotheses that VR may occur due to incomplete viral eradication,¹²
182 and supports studies to evaluate longer durations of N-R therapy.¹³

183
184 Finally, symptoms should not be relied upon to detect or exclude VR. Two individuals with VR
185 had a complete absence of symptoms during the VR period and less than half had symptom
186 rebound. Conversely, the majority of those who did have symptom rebound did not experience
187 VR.

188
189 Our study was limited by an observational design, with expected differences between those
190 taking N-R and untreated individuals based on treatment guidelines for N-R¹⁴. Nonetheless, VR
191 remained associated with N-R, even after adjustment for potential confounders. We used viral
192 culture as a surrogate for transmission risk but did not measure contagiousness or transmission
193 events directly.

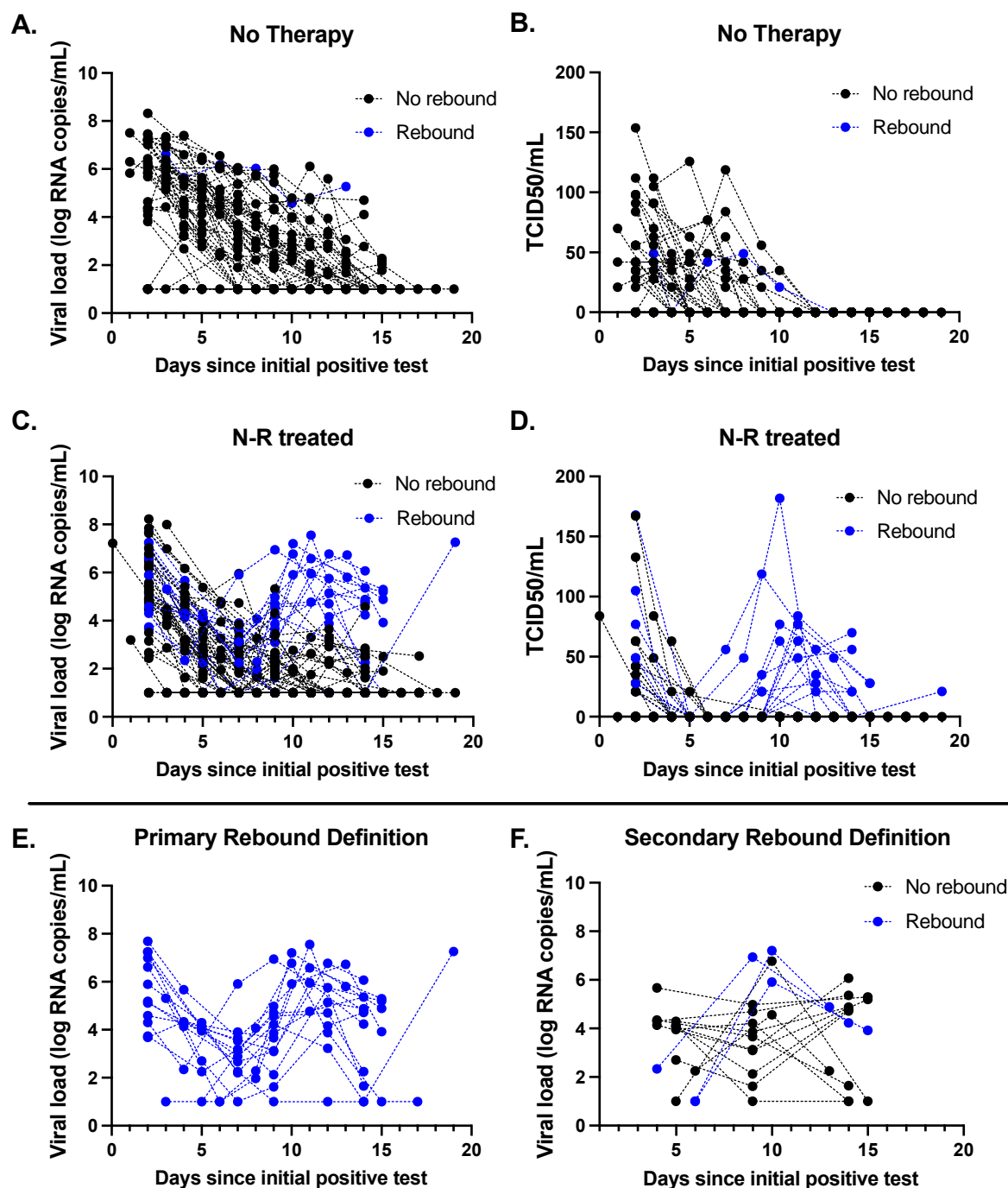
194
195 These data support a relationship between N-R use and VR. Future work should elucidate the
196 mechanistic pathways of VR, determine if delays in initiating N-R or longer courses of N-R may
197 prevent VR among high-risk individuals, and evaluate larger samples to identify the risk factors
198 for N-R-associated VR.

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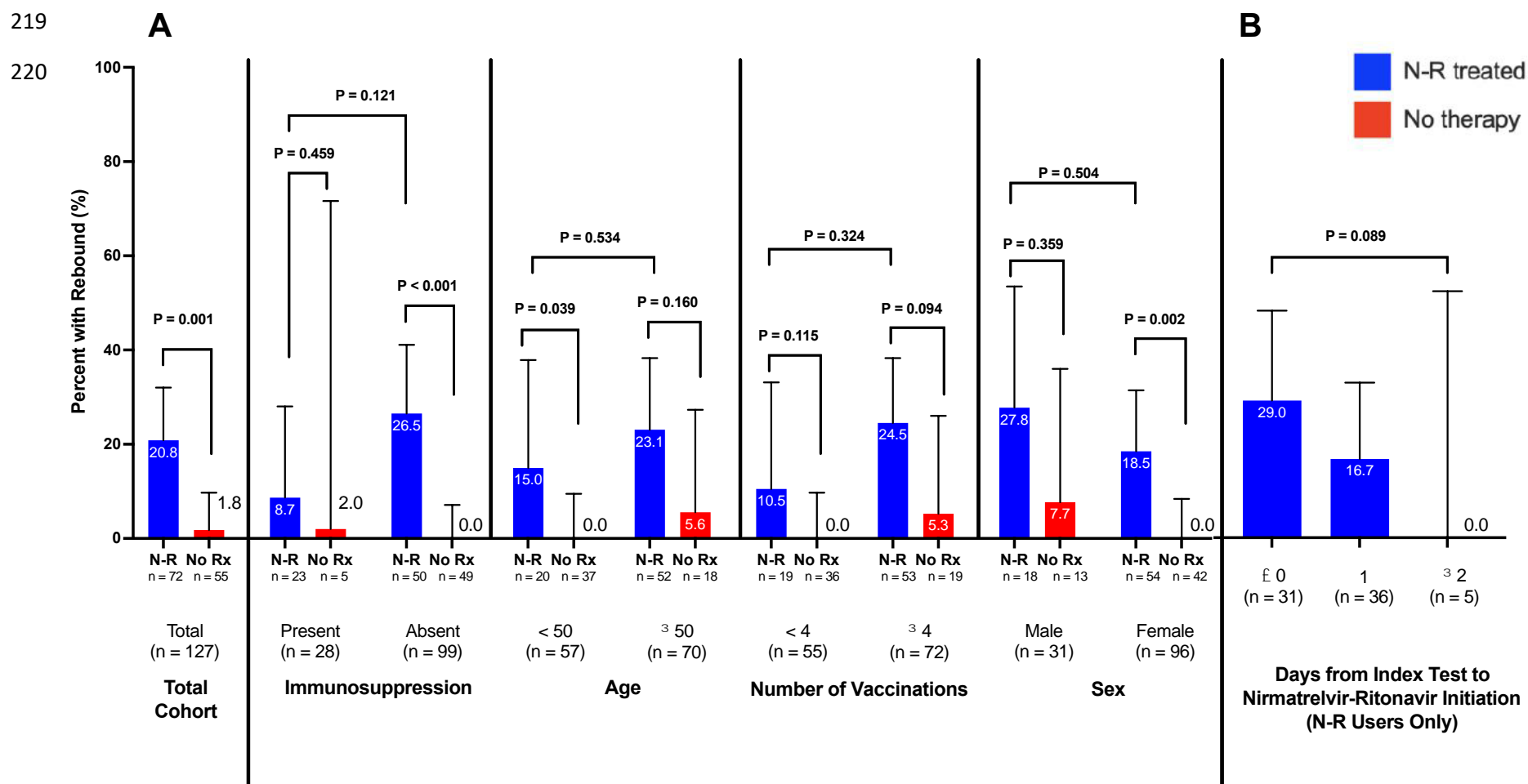
200 We would like to thank the study participants for their time and considerable efforts to provide
201 specimens in the acute phase of illness as part of this project.

202

Figure 1. Virologic decay curves with semiquantitative viral cultures and quantitative viral load among individuals with acute COVID-19 taking no therapy or nirmatrelvir-ritonavir (N-R). Black lines indicate individuals without rebound, whereas blue lines indicate individuals with virologic rebound. Panels A (viral load) and B (viral culture) depict decay curves for those not receiving therapy. Panels C (viral load) and D (viral culture) depict individuals who received N-R. Panels E and F compare our primary outcome with all available time points (E) or restricted to days 5, 10 and 14 only (Panel F) as defined in prior studies [1]. Using only three timepoints to detect rebound resulted in missing 81% of the observed virologic rebound events of replication-competent virus.

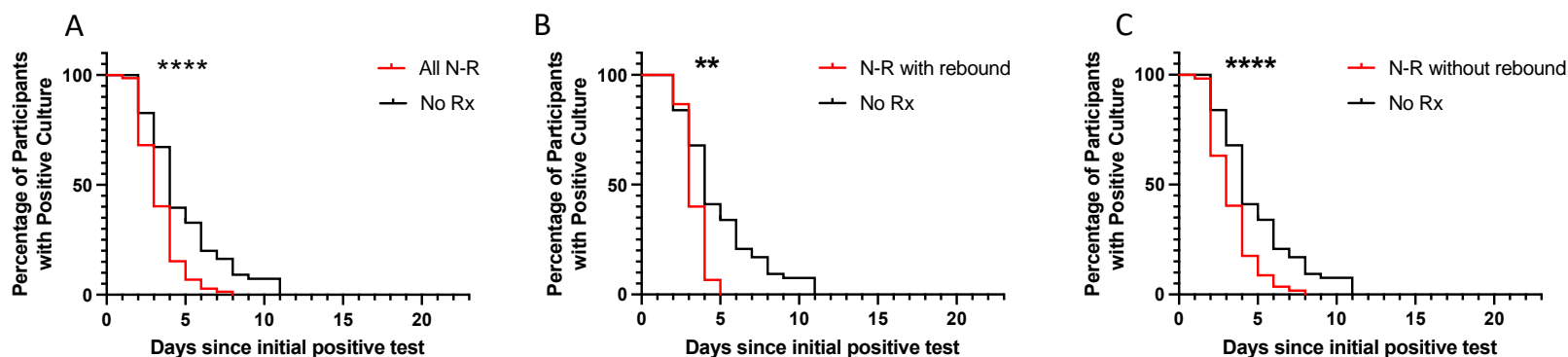


213 **Figure 2.** Comparative frequency of virologic rebound by nirmatrelvir-ritonavir use, stratified by demographics and clinical
 214 characteristics (A), and by number of days between the first positive SARS-CoV-2 test and initiation of nirmatrelvir-ritonavir therapy
 215 (B). For the sub-group comparisons, the bottom P-values represent Fisher's exact tests comparing rebound rates between those taking
 216 versus those not taking nirmatrelvir-ritonavir. The upper P-values represent Fisher's exact tests comparing rebound rates among those
 217 taking nirmatrelvir-ritonavir across the sub-groups, for example comparing those taking nirmatrelvir-ritonavir with
 218 immunosuppression present versus those taking nirmatrelvir-ritonavir with immunosuppression absent.

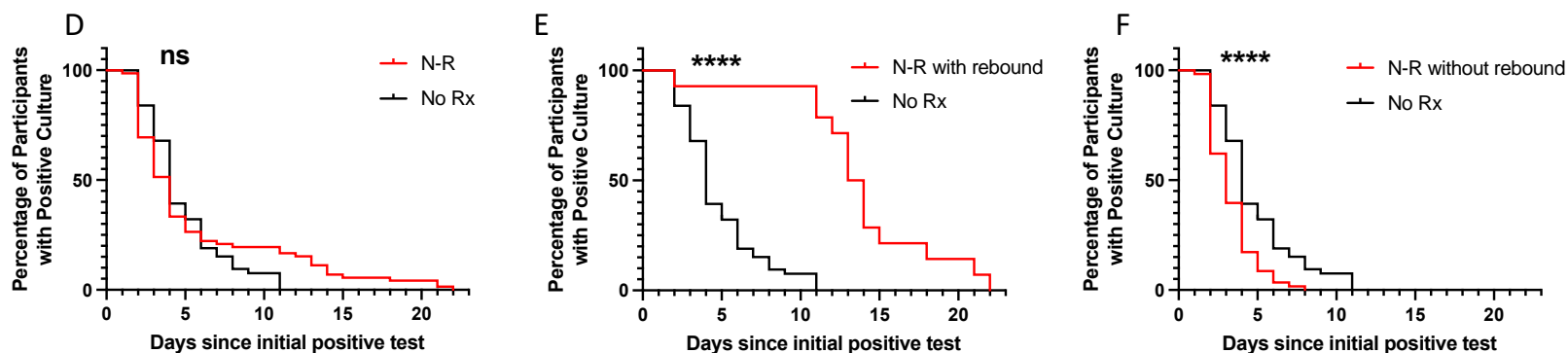


221 **Figure 3.** Kaplan Meier survival curves demonstrating time from initial positive SARS-CoV-2 test until initial negative viral culture
 222 (A-C) and final negative culture (D-F). In Panel A, we demonstrate that there is a faster time to first negative culture in those receiving
 223 nirmatrelvir-ritonavir (N-R) versus no therapy (No Rx). In Panels B and C, we find similar patterns in time to initial negative culture,
 224 when dividing the N-R group into those who rebounded (B) and those who did not (C). However, as shown in Panel D, there is no
 225 difference in time to final negative culture between N-R and No Rx groups. This appears to be due to the prolonged time to final
 226 negative culture among N-R users who rebound (Panel E), because the time to final negative culture remains shorter in N-R users who
 227 did not rebound compared to the No Rx group (Panel F).

Time to Initial Negative Culture (A-C)



Time to Final Negative Culture (D-F)



242 ns: non-significant; **: $P < 0.01$; ****: $P < 0.0001$

REFERENCES CITED

1. Anderson AS, Caubel P, Rusnak JM. Nirmatrelvir–ritonavir and viral load rebound in COVID-19. *New England Journal of Medicine*. 2022;387(11):1047-1049.
2. Charness ME, Gupta K, Stack G, et al. Rebound of SARS-CoV-2 Infection after Nirmatrelvir-Ritonavir Treatment. *N Engl J Med*. 2022;387(11):1045-1047. doi:10.1056/NEJMc2206449
3. Epling BP, Rocco JM, Boswell KL, et al. Clinical, Virologic, and Immunologic Evaluation of Symptomatic Coronavirus Disease 2019 Rebound Following Nirmatrelvir/Ritonavir Treatment. *Clin Infect Dis*. 2023;76(4):573-581. doi:10.1093/cid/ciac663
4. Pandit JA, Radin JM, Chiang D, et al. The COVID-19 Rebound Study: A Prospective Cohort Study to Evaluate Viral and Symptom Rebound Differences in Participants Treated with Nirmatrelvir Plus Ritonavir Versus Untreated Controls. *Clin Infect Dis*. Published online February 22, 2023:ciad102. doi:10.1093/cid/ciad102
5. Wong GLH, Yip TCF, Lai MSM, Wong VWS, Hui DSC, Lui GCY. Incidence of Viral Rebound After Treatment With Nirmatrelvir-Ritonavir and Molnupiravir. *JAMA Netw Open*. 2022;5(12):e2245086. doi:10.1001/jamanetworkopen.2022.45086
6. Deo R, Choudhary MC, Moser C, et al. Symptom and Viral Rebound in Untreated SARS-CoV-2 Infection. *Ann Intern Med*. 2023;176(3):348-354. doi:10.7326/M22-2381
7. Wong CKH, Lau KTK, Au ICH, et al. Viral burden rebound in hospitalised patients with COVID-19 receiving oral antivirals in Hong Kong: a population-wide retrospective cohort study. *Lancet Infect Dis*. Published online February 13, 2023:S1473-3099(22)00873-8. doi:10.1016/S1473-3099(22)00873-8
8. Boucau J, Uddin R, Marino C, et al. Characterization of Virologic Rebound Following Nirmatrelvir-Ritonavir Treatment for Coronavirus Disease 2019 (COVID-19). *Clin Infect Dis*. 2023;76(3):e526-e529. doi:10.1093/cid/ciac512
9. North CM, Barczak A, Goldstein RH, et al. Determining the Incidence of Asymptomatic SARS-CoV-2 Among Early Recipients of COVID-19 Vaccines (DISCOVER-COVID-19): A Prospective Cohort Study of Healthcare Workers Before, During and After Vaccination. *Clin Infect Dis*. 2022;74(7):1275-1278. doi:10.1093/cid/ciab643
10. Goyal A, Reeves DB, Cardozo-Ojeda EF, Schiffer JT, Mayer BT. Viral load and contact heterogeneity predict SARS-CoV-2 transmission and super-spreading events. Walczak AM, Childs L, Forde J, eds. *eLife*. 2021;10:e63537. doi:10.7554/eLife.63537
11. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature*. 2020;581(7809):465-469. doi:10.1038/s41586-020-2196-x
12. Perelson AS, Ribeiro RM, Phan T. *An Explanation for SARS-CoV-2 Rebound after Paxlovid Treatment*. Infectious Diseases (except HIV/AIDS); 2023. doi:10.1101/2023.05.30.23290747

13. National Institutes of Health. A Study to Learn About the Study Medicines (Nirmatrelvir Plus Ritonavir) in People Aged 12 Years or Older With COVID-19 and a Compromised Immune System (NCT05438602). Published April 11, 2023. Accessed April 20, 2023. <https://clinicaltrials.gov/ct2/show/NCT05438602>
14. National Institutes of Health. Therapeutic Management of Nonhospitalized Adults With COVID-19. COVID-19 Treatment Guidelines. Published April 20, 2023. Accessed April 21, 2023. <https://www.covid19treatmentguidelines.nih.gov/management/clinical-management-of-adults/nonhospitalized-adults--therapeutic-management/>